Published in final edited form as:

Head Neck. 2014 December ; 36(12): 1747–1753. doi:10.1002/hed.23535.

The association between severe treatment-related lymphopenia and progression free survival in patients with newly diagnosed squamous cell head and neck cancer

Jian Campian, MD, PhD¹, Guneet Sarai, MD², Xiaobu Ye, MD, MS¹, Shanthi Marur, MD¹, and Stuart A Grossman, MD¹

¹Departments of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD

²Good Samaritan Hospital, Baltimore, MD

Abstract

Background—Severe treatment-related lymphopenia (TRL) occurs commonly in many cancers and is associated with early tumor progression. Data is lacking as to whether this occurs in squamous cell head/neck cancer.

Methods—Serial total lymphocyte counts (TLC) were retrospectively reviewed in patients with newly diagnosed squamous head and neck cancer undergoing chemoradiation and associated with treatment outcomes.

Results—The median baseline TLC in 56 patients was 1660 cells/mm³ which fell by 73% to 445 cells/mm³ two months after initiating chemoradiation ($p < 0.0001$). HPV– patients with TLC < 500 cells/mm³ at two months had significantly earlier disease progression than those with higher TLCs (HR 5.75, $p = 0.045$).

Conclusions—Baseline TLCs were normal but at two months ~60% of patients had severe TRL regardless of HPV status. Severe TRL in HPV– patients is independently associated with earlier disease progression. Prospective studies are needed to confirm these findings which suggest that immune preservation is important in this cancer.

Keywords

Lymphopenia; Head and neck squamous cell carcinoma; Radiation; Chemotherapy; Treatment-related toxicities

INTRODUCTION

The immune system has long been thought to be important in the prevention and control of cancers. As a result, decades of research have been devoted to developing vaccines and other immune approaches for cancer therapy. The human immune system has several mechanisms for identifying cancer cells and removing them from the body, most of which

Corresponding Author: Stuart A. Grossman, MD, Professor of Oncology, Medicine & Neurosurgery, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Suite 1M-16, 1550 Orleans Street, Baltimore, MD 21287, Phone: 410-955-8837 Fax: 410-614-9335, grossman@jhmi.edu.

involve lymphocytes. Lymphocytes comprise about 30% of the normal human white blood cell population and are essential effector cells in the immune response to cancer.¹ The presence of adequate populations of circulating lymphocytes appears to be correlated with the efficacy of pharmacologic antitumor immune modulators, such as ipilimumab.² Cancer vaccines, such as recently approved sipuleucel-T (Provenge), also rely on lymphocytes to be effective.^{3, 4}

Pre-treatment lymphopenia has been documented to be a poor prognostic factor in patients with cancers of the lung, breast, colorectum as well as sarcomas, and lymphomas.^{5, 6} In addition, pathologic studies have found that patients with intense lymphocytic infiltration of their solid tumors have longer survival rates than those who do not.^{7–10}

Head and neck squamous cell carcinoma (HNSCC) is an immunogenic tumor as shown by a variable amount of infiltrating lymphocytes and other immune cells.^{11, 12} It has been reported that patients with HNSCC have significantly lower absolute numbers of CD3+, CD4+ and CD8+ T cells than normal controls.¹³ In addition, CD3+ and CD4+ T-cell counts appear to be significantly lower in patients with active disease than in those with no evidence of disease.¹³ Other previous studies have suggested that pretreatment lymphocyte counts are associated with prognosis in HNSCC.¹⁴

Although it has been previously documented that pre-treatment lymphopenia is associated with poor prognosis, only recently has post-treatment lymphopenia been associated with inferior survival outcomes.^{15–17} The first documentation of this association was in a prospective study that followed serial CD4 counts in 96 patients with newly diagnosed high grade gliomas.¹⁶ Although these patients began with normal CD4 counts, severe lymphopenia (CD4 counts <200 cells/mm³) occurred in 40% of patients 10 weeks after beginning concurrent radiation and temozolomide, and this lymphopenia was long-lasting. Multivariate analysis revealed that this treatment-related lymphopenia was independently associated with shorter overall survival. These patients died of progressive tumor and not opportunistic infections. A recent report of patients with resectable pancreatic adenocarcinoma found that this patient population had normal lymphocyte counts following surgery but two months after the initiation of radiation and chemotherapy 45% of patients had developed severe lymphopenia.¹⁵ Multivariate analysis revealed that this severe lymphopenia was also independently associated with early death from progressive cancer. Similarly, 50% of patients with stage III NSCLC treated with concurrent chemotherapy and radiation developed severe lymphopenia and those patients died early of progressive cancer. Multivariate analysis revealed an association between severe TLC and survival (HR 1.70, 95% CI: 0.8–3.6).¹⁷ A recent paper suggests that local radiation may play a primary role in the etiology of treatment-related lymphopenia.¹⁸

This retrospective study was performed to determine if patients with newly diagnosed HNSCC also develop significant lymphopenia after concurrent radiation and chemotherapy and if this treatment-related lymphopenia is associated with early tumor recurrence and reduced survival in this patient population.

PATIENTS AND METHODS

Study population

This study was reviewed and approved by the Institutional Review Board of the Johns Hopkins University. Patients were retrospectively identified using a database of HNSCC patients at the Johns Hopkins Hospital. The following eligibility criteria were required: 1) newly diagnosed HNSCC between 2007 and 2009, 2) 18 years of age, 3) received concurrent platinum-based chemoradiation at our institution, 4) Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, and 5) baseline and follow-up complete blood counts performed at Johns Hopkins Hospital and thus accessible through the Electronic Patient Record (EPR). Patients were excluded if they had received chemotherapy or radiation prior to their HNSCC treatment.

Treatment and total lymphocyte counts examination

Information relating to known prognostic factors in HNSCC was obtained from the medical records of each patient.^{19, 20} Baseline values collected prior to initiation of radiation and chemotherapy included: complete blood count (CBC), total lymphocyte counts, Human papillomavirus (HPV) status, ECOG performance status, smoking history, alcohol dependence, T staging, N staging and overall clinical staging, and primary tumor site. In addition, the dose and duration of radiation, whether the chemotherapy regimen was cisplatin or carboplatin based, and treatment responses were collected. CBC's were routinely performed monthly after radiation and chemotherapy were initiated and these values were also recorded.

Total lymphocyte counts were collected before beginning chemoradiation and monthly thereafter for a total of 24 months. Baseline TLCs were classified as normal (≥ 1000 cells/mm³) or abnormal (<1000 cell/mm³). Following the initiation of antineoplastic treatment, the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0) was used to classify the severity of treatment related lymphopenia.

The TLCs at two months after the initiation of radiation and chemotherapy were dichotomized to CTCAE grade 0–II versus grade III–V for the relevant analyses. For patients with missing lymphocyte counts at 2 months, the TLCs at 1 month were used instead. Progression free survival (PFS) was measured from the date of diagnosis to the date of tumor recurrence or censored at the date of last follow-up. Overall survival time was measured from the date of diagnosis to the date of death due to any cause. Survival was censored if the subject was alive at the time of last follow-up.

Statistical analysis

Patient baseline characteristics were summarized using descriptive statistics. Chi-square test statistics were used for proportional comparison. Student's t-test and paired t-test were used for continuous data between and within group comparison, respectively. Survival probability was estimated using the Kaplan-Meier method.²¹ The confidence interval of median survival time was constructed by the method of Brookmeyer-Crowley.²² Univariate analysis was

used to assess an association between potential prognostic factors and PFS. For patients who were HPV negative, their important baseline and disease characteristics associated with PFS were identified in the univariate analysis using a p value of 0.25 for constructing the multivariate proportional hazards regression model. The proportional hazards regression model was used to estimate the hazard ratio (HR) for tumor recurrence attributable to prognostic factors. All p values are reported as 2-sided and all analyses were conducted using SAS software (version 9.2, SAS Institute).

RESULTS

Baseline characteristics of patients

Fifty-six adults met the required eligibility criteria. Baseline demographic information on these patients is provided in Table 1. The median age of the patients was 57 years (range 37–77) and 63% of the patients were over the age of 55. Seventy-nine percent were male, 71% were Caucasian, and 88% had an ECOG performance status of zero. Sixty-one percent were HPV positive. Fifty-nine percent had smoked more than 10 pack years. Eleven percent had documented alcohol dependence. Sixteen percent had surgery prior to concurrent chemoradiation. Three patients (5%) were HIV positive and on antiviral therapy. Each of these patients had normal pre-treatment total lymphocyte counts and received standard chemoradiation. Seventy percent of patients had an oropharyngeal tumor. Twenty-three percent were stage I–III and 77% were stage IVA or IVB. Forty percent were T classification 3–4 and 56% were N classification 2B–3. Cisplatin was administered as concurrent chemotherapy in 95% of patients. Eighty-eight percent had complete or partial responses (Table 1).

Total lymphocyte counts over time

The median total lymphocyte count (TLC) for all patients prior to chemoradiation was 1660 cells/mm³ (range 890–3090 cells/mm³) and only three patients (5%) had baseline TLC <1000 cells/mm³ (890, 940, and 970 cells/mm³). Two months after beginning radiation with cisplatin (n=53) or carboplatin (n=3) the TLC fell by 73% with 61% patients having TLC <500 cells/mm³ (median 445 cells/mm³, p<0.0001) (Figure 1). In addition, as shown in Figure 1, the median TLC remained below 1000 cells/mm³ for the entire one year observation period. Nine patients had missing lymphocyte counts at 2 months and in these patients the TLCs at 1 month were used.

As noted in Table 1, there was no significant difference in the baseline demographic, staging, laboratory, or adjuvant treatment data in patients whose TLCs remained above 500 cells/mm³ or fell significantly at 2 months after chemoradiation treatment.

Total lymphocyte counts with HPV status

For purposes of analysis, patients were divided into two groups depending on HPV status. The median TLC dropped significantly with chemoradiation in both HPV+ and HPV– patients. HPV+ patients (n=34) had baseline median TLC of 1610 cells/mm³ which fell to 320 cells/mm³ 2 months after beginning chemoradiation. A total of 26 patients (76%) had TLC <500 cells/mm³ at 2 months. HPV– (n=22) patients started with a median TLC at 1770

cells/mm³ which fell to 550 cells/mm³ with 8 patients (36%) having TLC <500 cells/mm³ 2 months after beginning chemoradiation (Figure 2).

Tumor recurrence

Overall 14 of the 56 patients (25%) had recurrence of tumor and 9 patients (16%) died of their tumor. HPV+ patients had significantly longer overall survival (OS) ($p=0.006$) and PFS ($p=0.01$) than HPV- patients (Figure 3A and 3B). Five of 34 HPV+ patients had tumor progression and 2 have died while 9/22 HPV- patients had disease progression and 7 died. HPV- patients who developed grade III-IV treatment-related lymphopenia (TLC <500 cells/mm³) two months after beginning radiation and chemotherapy had a significant higher hazard rate of disease progression than those whose TLC remained higher (Figure 3C).

Observed infections

Total of six patients (11%) had a documented infection within the 12-month observation period after the initiation of chemoradiation. Four of these were HPV-. Five patients had post-operative MRSA infections and recovered uneventfully with intravenous vancomycin. One patient had cellulitis which rapidly resolved with oral antibiotics. Five of these six patients with infections are alive and one died from tumor progression approximately two years after diagnosis. The three HIV positive patients did not develop infections during their one year follow-up period.

Baseline characteristics of HPV- patients

Baseline demographic information on HPV- patients is provided in Table 2. The median age of the patients was 60 years (range 37-77) and 68% of the patients were over the age of 55. Sixty-eight percent were male, 45% were Caucasian, and 73% had an ECOG performance status of zero. Seventy-seven percent had smoked more than 10 pack years. All three HIV patients were HPV-. Forty-one percent were stage I-III and 59% were stage IVA or IVB. Fifty-seven percent were T classification 3-4 and 43% were N classification 2B-3. Twenty-three percent patients had an oropharyngeal tumor. Sixty-eight percent had complete or partial responses (Table 2). The median total lymphocyte count (TLC) for all HPV- patients prior to chemoradiation was 1770 cells/mm³ (range 940-3090 cells/mm³) with 91% had baseline TLC > 1000 cells/mm³. Pre-treatment lymphopenia (TLC <1000 cells/mm³) was present in only two patients (9%). As noted in Table 2, these HPV- patients were well balanced at the baseline demographic, staging, laboratory, or adjuvant treatment data in whose TLCs remained above 500 cells/mm³ or fell significantly at 2 months after chemoradiation treatment.

Associations between patient characteristics and progression-free survival in HPV- patients

Table 3 presents univariate and multivariate associations between patient characteristics and progression-free survival among 22 HPV- patients. No significant univariate associations were identified between potential prognostic factors and PFS. Multivariate analysis revealed a strikingly higher hazard of disease progression for HPV- patients who developed grade III-V TRL (TLC <500 cells/mm³) two months after beginning radiation and chemotherapy

(HR 5.75, 95%CI: 1–32; $p=0.045$) adjusting for tumor site, T classification and smoking history (Table 3). Too few events occurred in the HPV+ patients for analysis. All three of the HIV positive patients remain alive without evidence of recurrence. Two of the 22 HPV– patients had mild pre-treatment lymphopenia. One remains without recurrence and the other died from tumor progression two years after diagnosis. As there have only been two deaths (5%) in the HPV+ patients and seven deaths (31%) in HPV– patients, there are too few events to formally evaluate correlations between TLC and survival.

DISCUSSION

In this retrospective analysis of patients with newly diagnosed HNSCC median TLCs were normal prior to the initiation of radiation and chemotherapy. However, two months after beginning chemoradiation, TLCs fell in ~60% of patients to levels associated with severe immunosuppression regardless of their HPV status. Furthermore, this lymphopenia did not return to baseline levels during 12 months of observation. The timing, frequency, severity and duration of this treatment-induced lymphopenia is comparable to that recently reported in patients with newly diagnosed high grade gliomas, resected pancreatic cancer, and stage III non-small cell lung cancers after treatment with chemoradiation.^{15–17}

The association of TRL with PFS and overall survival is also similar in patients with head and neck cancer to that reported in other cancers treated with radiation therapy. No significant difference was observed in overall survival or PFS between patients with $TLC < 500$ cells/mm³ and those with $TLC \geq 500$ cells/mm³ for all study subjects including both HPV+ and HPV– patients (data not shown). This is likely due to the fact that HPV+ patients have a more favorable prognosis, obscuring any effect on PFS in the general group. However, when patients were divided into two groups based on their HPV status, not surprisingly, there was better overall survival and PFS in HPV+ group compared to HPV– group. More importantly, HPV– patients who developed severe lymphopenia ($TLC < 500$ cells/mm³) two months after beginning chemoradiation had early tumor recurrence and multivariate analysis revealed a strikingly higher hazard rate for disease progression than those whose $TLC \geq 500$ cells/mm³ (HR 5.75, 95%CI: 1–32; $p=0.045$). We could not conduct this analysis in the HPV+ cohort since very few HPV+ patients progressed. No significant difference was noticed in overall survival between patients with $TLC < 500$ cells/mm³ and $TLC \geq 500$ cells/mm³ in either HPV+ or HPV– cohort (data not shown). This is likely due to the relatively short follow-up and small sample size in this study.

Several studies published in the 1970's documented an association between radiation and lymphocyte counts in a variety of different cancers including HNSCC.^{23–25} More recent studies have also confirmed these findings. Tisch et al reported that during radiation treatment, the total lymphocyte counts, CD8+ T lymphocyte counts, and especially CD4+ T lymphocyte counts decreased significantly in patients with HNSCC. The mean CD4 counts dropped from 739 cells/mm³ to 183 cells/mm³ and the CD4+/CD8+ ratio also decreased significantly. Not only the lymphocyte counts but also the in vitro lymphocyte stimulation responses decreased.²⁶ Study by Yang et al also reported a decreased percentage of CD4+ T cells and a reduced ratio of CD4+/CD8+ in patients with head and neck cancer after radiation therapy.²⁷ Another study by Gray et al reported that the patients with head and

neck cancer who received radiation therapy had a significant decrease in total lymphocytes and lymphocyte subsets. The effects were also prolonged in duration. Significant decreases in lymphocyte subsets were not found in patients tested before treatment or in patients treated with surgery alone.²⁴ Other investigators have noted that CD4+ T cells but not CD8+ T cells appeared to be affected by previous radiotherapy in HNSCC, and the CD4+ T cell counts remained decreased for long period of time.^{28, 13} The CD4+ T cell counts was not recovered for 2 years in patients who had no evidence of disease after surgery and radiation for their HNSCC.¹³ A previous study indicated that disturbed lymphocyte homeostasis might be especially prominent in patients with poor prognosis stage IV disease and nodal metastases.¹³

The most interesting finding of the study reported in this manuscript is the observation that grade III–IV treatment related lymphopenia is associated with early tumor recurrence in patients with HPV– squamous cell cancers of the head and neck. There is one previously published study which suggested that radiation induced lymphopenia was associated with a predisposition to tumor recurrence.²⁹ These results mirror those seen in patients with high grade gliomas, pancreatic cancer, and stage III non-small cell lung cancer and appear likely to result from radiation to circulating lymphocytes regardless of the location of the radiation field.^{15–17} We have recently demonstrated that neo-adjuvant chemotherapy in patients with stage III non-small cell lung cancer does not result in severe lymphopenia but that this routinely occurs following the administration of local radiation therapy.¹⁷ In addition, we have recently shown that local radiation provides potentially toxic doses of radiation to over 90% of circulating lymphocytes in patients with primary brain tumors once again suggesting that post-treatment lymphopenia occurs primarily as a result of radiation of circulating lymphocytes.¹⁸

Our PFS findings in HPV– patients raise the possibility that the status of the immune system may play a significant role in prognosis in this poor risk group where current treatment is less effective. This could also be true in HPV+ patients, but as this disease has a much better overall prognosis a much larger sample size and longer follow-up would be required to obtain sufficient progression or survival events for this to be identifiable. Alternatively, because HPV+ cancers are much more sensitive to therapy, it may be that the status of immune system is not as important to survival outcomes as it is in HPV– patients. While radiation had very similar effect on total lymphocyte counts in both HPV+ and HPV– patients, it is not clear what differences there are in the T lymphocyte subsets of these two patient populations. Additional information on the radiosensitivity of different lymphocyte subtypes (ie primed memory immune T cells and T regulatory cells) could be extremely important to investigators integrating HPV vaccine into therapy in patients with head and neck cancer and should be a focus of future research.

Our findings strongly suggest that treatment related lymphopenia is common, severe and long lasting in patients with HNSCC and that it is associated with early disease progression in poor risk patients. However, there are several substantial limitations to our study which should be carefully considered. This is a small, retrospective study with a limited number of progression and death events. Our observations do not answer mechanistic questions. For example, the retrospective nature of this study lacks standardization of chemotherapy doses

or timing, measurement of potential biologic markers of chemosensitivity, and prospective recording of side effects or toxicities. However, our findings emphasize the importance of these findings and set the stage for much needed prospective studies focusing on these issues in patients with HNSCC. As a result, larger prospective studies are needed not only to confirm these findings, also to address the possible underlying mechanisms that might link treatment-related lymphopenia to PFS or overall survival. Similar findings in many different cancers make it likely that novel approaches to protect the immune system during radiation and chemotherapy or to reconstitute it after therapy may be of vital importance in improving PFS and overall survival especially in patients with relatively unresponsive cancers.

Acknowledgments

Sources of support: None

References

1. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011; 480:480–489. [PubMed: 22193102]
2. Ku GY, Yuan J, Page DB, et al. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. *Cancer*. 2010; 116:1767–1775. [PubMed: 20143434]
3. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010; 363:411–422. [PubMed: 20818862]
4. Huber ML, Haynes L, Parker C, Iversen P. Interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. *J Natl Cancer Inst*. 2012; 104:273–279. [PubMed: 22232132]
5. Lissoni P, Brivio F, Fumagalli L, et al. Efficacy of cancer chemotherapy in relation to the pretreatment number of lymphocytes in patients with metastatic solid tumors. *Int J Biol Markers*. 2004; 19:135–140. [PubMed: 15255546]
6. Ray-Coquard I, Cropet C, Van Glabbeke M, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res*. 2009; 69:5383–5391. [PubMed: 19549917]
7. Hiraoka K, Miyamoto M, Cho Y, et al. Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. *Br J Cancer*. 2006; 94:275–280. [PubMed: 16421594]
8. Lohr J, Ratliff T, Huppertz A, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. *Clin Cancer Res*. 2011; 17:4296–4308. [PubMed: 21478334]
9. Dahlin AM, Henriksson ML, Van Guelpen B, et al. Colorectal cancer prognosis depends on T-cell infiltration and molecular characteristics of the tumor. *Mod Pathol*. 2011; 24:671–682. [PubMed: 21240258]
10. Swann JB, Smyth MJ. Immune surveillance of tumors. *J Clin Invest*. 2007; 117:1137–1146. [PubMed: 17476343]
11. Wolf GT, Hudson JL, Peterson KA, Miller HL, McClatchey KD. Lymphocyte subpopulations infiltrating squamous carcinomas of the head and neck: correlations with extent of tumor and prognosis. *Otolaryngol Head Neck Surg*. 1986; 95:142–152. [PubMed: 2954014]
12. Whiteside TL, Chikamatsu K, Nagashima S, Okada K. Antitumor effects of cytolytic T lymphocytes (CTL) and natural killer (NK) cells in head and neck cancer. *Anticancer Res*. 1996; 16:2357–2364. [PubMed: 8816835]
13. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. *Clin Cancer Res*. 2004; 10:3755–3762. [PubMed: 15173082]

14. Wolf GT, Schmaltz S, Hudson J, et al. Alterations in T-lymphocyte subpopulations in patients with head and neck cancer. Correlations with prognosis. *Arch Otolaryngol Head Neck Surg.* 1987; 113:1200–1206. [PubMed: 3499156]
15. Balmanoukian A, Ye X, Herman J, Laheru D, Grossman SA. The association between treatment-related lymphopenia and survival in newly diagnosed patients with resected adenocarcinoma of the pancreas. *Cancer Invest.* 2012; 30:571–576. [PubMed: 22812722]
16. Grossman SA, Ye X, Lesser G, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. *Clin Cancer Res.* 2011; 17:5473–5480. [PubMed: 21737504]
17. Campian JL, Ye X, Brock M, Grossman SA. Treatment-related lymphopenia in patients with stage III non-small cell lung cancer. *Cancer Invest.* 2013; 31:183–188. [PubMed: 23432821]
18. Yovino S, Kleinberg L, Grossman SA, Narayanan M, Ford E. The etiology of treatment-related lymphopenia in patients with malignant gliomas: modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. *Cancer Invest.* 2013; 31:140–144. [PubMed: 23362951]
19. De Castro G Jr, Pasini FS, Siqueira SA, et al. ERCC1 protein, mRNA expression and T19007C polymorphism as prognostic markers in head and neck squamous cell carcinoma patients treated with surgery and adjuvant cisplatin-based chemoradiation. *Oncol Rep.* 2011; 25:693–699. [PubMed: 21206986]
20. Kokemueller H, Brachvogel P, Eckardt A, Hausamen JE. Neck dissection in oral cancer--clinical review and analysis of prognostic factors. *Int J Oral Maxillofac Surg.* 2002; 31:608–614. [PubMed: 12521316]
21. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958; 53:457–481.
22. Brookmeyer R, Crowley J. A Confidence Interval for the Median Survival Time. *Biometrics.* 1982; 38:29–41.
23. Nordman E, Toivanen A. Effects of irradiation on the immune function in patients with mammary, pulmonary or head and neck carcinoma. *Acta Radiol Oncol Radiat Phys Biol.* 1978; 17:3–9. [PubMed: 696399]
24. Gray WC, Chretien PB, Suter CM, et al. Effects of radiation therapy on T-lymphocyte subpopulations in patients with head and neck cancer. *Otolaryngol Head Neck Surg.* 1985; 93:650–660. [PubMed: 2932670]
25. Stratton JA, Byfield PE, Byfield JE, Small RC, Benfield J, Pilch Y. A comparison of the acute effects of radiation therapy, including or excluding the thymus, on the lymphocyte subpopulations of cancer patients. *J Clin Invest.* 1975; 56:88–97. [PubMed: 1095613]
26. Tisch M, Heimlich F, Daniel V, Opelz G, Maier H. Cellular immune defect caused by postsurgical radiation therapy in patients with head and neck cancer. *Otolaryngol Head Neck Surg.* 1998; 119:412–417. [PubMed: 9782004]
27. Yang SJ, Rafla S, Youssef E, Selim H, Salloum N, Chuang JY. Changes in T-cell subsets after radiation therapy. *Radiology.* 1988; 168:537–540. [PubMed: 3260678]
28. Friedman EJ. Immune modulation by ionizing radiation and its implications for cancer immunotherapy. *Curr Pharm Des.* 2002; 8:1765–1780. [PubMed: 12171547]
29. Verastegui EL, Morales RB, Barrera-Franco JL, Poitevin AC, Hadden J. Long-term immune dysfunction after radiotherapy to the head and neck area. *Int Immunopharmacol.* 2003; 3:1093–1104. [PubMed: 12860166]

Figure 1.

Treatment-related lymphopenia is common and severe in newly diagnosed HNSCC. Median total lymphocyte counts (TLCs) over 12-month period after initiation of chemo/RT were analyzed (n=56). TLCs were normal prior to chemo/RT. However, 2 months after initiation of concurrent chemo/RT, TLCs fell rapidly by 73% with 61% patients having TLC <500 cells/mm³. TLCs remained below 1000 cells/mm³ throughout the observation period. Gray arrow depicts the start of radiation exposure and chemotherapy. Abbreviations: TLC, total lymphocyte count; RT, radiation.

Figure 2.

Treatment-related lymphopenia is seen in both HPV+ and HPV- patients. Median total lymphocyte counts dropped significantly with the chemo/RT in both HPV+ and HPV- patients. (A) HPV+ patients (n=34) had baseline median TLC of 1610 cells/mm³ which fell to 320 cells/mm³ 2 months after beginning chemo/RT. A total of 26 patients (76%) had TLC <500 cells/mm³ at 2 months. (B) HPV- (n=22) patients started with a median TLC at 1770 cells/mm³ which fell to 550 cells/mm³ with 8 patients (36%) having TLC <500 cells/mm³ 2 months after beginning chemo/RT.

Abbreviations: TLC, total lymphocyte count; RT, radiation; HPV, Human papillomavirus.

Figure 3.

Severe treatment-related lymphopenia in HPV– patients is associated with early tumor recurrence. (A) Kaplan-Meier survival curves for patients with HPV positive patients (above red line) and HPV negative patients (below green line). The median overall survival in HPV + patients is significantly longer compared to HPV– patients ($p=0.006$). (B) The progression-free survival in HPV+ patients is also significantly longer compared to HPV– patients ($p=0.01$). (C) HPV– patients who developed severe treatment-related lymphopenia ($TLC < 500$ cells/mm³) 2 months after beginning chemo/RT had a strikingly higher hazard rate for disease progression than those whose TLC remained higher (multivariate analysis HR 5.75, 95%CI: 1–32; $p=0.045$).

Abbreviations: TLC, total lymphocyte count; RT, radiation; HPV, Human papillomavirus.

Table 1

Baseline Characteristics of All Patients and Those with Lymphocyte Counts Above and Below 500 cells/mm³ at 2 Months

	All patients (N=56)	Patients with lymphocyte counts ≥500 at 2 months (N=22)	Patients with lymphocyte counts <500 at 2 months (N=34)	P-value
Demographic Data				
Age: median (range)	57 (37–77)	56 (46–77)	57.5 (37–74)	0.37
Male # (%)	44 (79)	18 (82)	26 (76)	0.64
Caucasian: # (%)	40 (71)	11 (50)	29 (85)	0.004
HPV positive: # (%)	34 (61)	8 (36)	26 (76)	0.027
ECOG performance =0: # (%)	49 (88)	17 (77)	32 (94)	0.06
Smoking>10 pack-year: # (%)	33 (59)	16 (73)	17 (50)	0.09
ETOH depend: # (%)	6 (11)	3 (14)	3 (9)	0.57
Baseline laboratory data				
Total lymphocyte count: median (range)	1660 (890–3090)	1945 (940–3090)	1505 (890–2860)	0.04
Lymphocyte 1000: # (%)	53 (95)	20 (91)	33 (97)	0.32
WBC: median (range)	6665 (3810–19800)	7040 (5310–19800)	6535 (3810–9940)	0.20
Hematocrit: median (range)	41.3 (27.1–47.3)	40.2 (27.1–47.2)	41.1 (31.8–47.3)	0.43
Platelet: median (range)	251 (148–589)	234 (162–448)	261 (148–589)	0.25
Tumor staging data				
T stage 3–4: # (%)	22 (40)	12 (55)	10 (70)	0.07
N stage 2B-3: # (%)	31 (56)	13 (59)	18 (55)	0.74
Overall stage I–III: # (%)	13 (23)	5 (23)	8 (24)	0.94
Oropharyngeal tumor: # (%)	39 (70)	11 (50)	28 (82)	0.01
Surgical resected: # (%)	9 (16)	6 (27)	3 (9)	0.07
Treatment data				
Concurrent chemotherapy (Cisplatin): # (%)	53 (95)	20 (91)	33 (97)	0.32
Complete and partial response: # (%)	49 (88)	19 (86)	30 (88)	0.84

Table 2

Baseline Characteristics of HPV negative Patients and Those with Lymphocyte Counts Above and Below 500 cells/mm³ at 2 Months

	All patients (N=22)	Patients with lymphocyte counts 500 at 2 months (N=14)	Patients with lymphocyte counts <500 at 2 months (N=8)
Demographic Data			
Age: median (range)	60 (37–77)	60 (51–77)	57 (37–72)
Male # (%)	15 (68)	11 (79)	4 (50)
Caucasian: # (%)	10 (45)	5 (36)	5 (63)
ECOG performance =0: # (%)	16 (73)	10 (71)	6 (75)
Smoking>10 pack-year: # (%)	17 (77)	11 (79)	6 (75)
HIV positive: # (%)	3 (14)	2 (14)	1 (13)
Baseline laboratory data			
Total lymphocyte count: median (range)	1770 (940–3090)	1830 (940–3090)	1655 (1220–2860)
Lymphocyte <1000: # (%)	2 (9)	2 (100)	0 (0)
Tumor staging data			
T stage 3–4: # (%)	12 (57)	9 (64)	3 (43)
N stage 2B-3: # (%)	9 (43)	6 (43)	3 (43)
Overall stage I–III: # (%)	9 (41)	4 (29)	5 (63)
Oropharyngeal tumor: # (%)	5 (23)	3 (21)	2 (25)
Treatment data			
Complete and partial response: # (%)	15 (68)	11 (78)	4 (50)
Incidence of infection: # (%)	4 (18)	3 (21)	1 (13)

Table 3

Univariate and Multivariate Associations between Patient Characteristics and Progression-Free Survival in HPV Negative Patients

Characteristic	Hazard Ratio (95% CI)	p-value
Univariate Associations		
Age	0.98 (0.91–1.06)	0.652
ECOG	0.49 (0.10–2.40)	0.383
T Stage: 0–2 vs. 3–4	0.36 (0.07–1.77)	0.207
Tumor site: OP vs. non-OP	0.29 (0.04–2.37)	0.248
Smoking: no smoke vs. smoke	1.02 (0.24–4.28)	0.978
Lymph count at 2 months: <500 vs. 500*	2.61 (0.65–10.52)	0.177
Multivariate Associations		
Lymph count at 2 months: <500 vs. 500	5.74 (1.04–31.59)	0.045
Tumor site: OP vs. non-OP	0.20 (0.02–2.01)	0.171
T stage: 0–2 vs. 3–4	0.278 (0.05–1.67)	0.161

* Lymphocyte count at 2 months is dichotomized at 500 cells/mm³ (per the CTCAE NIH grade III–IV treatment induced lymphopenia)